

# Draft NTP Technical Report TR570 on $\alpha,\beta$ -Thujone

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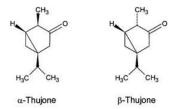


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## **Nomination**



- Thujone is a monoterpene ketone found in several plant species
- It exists in nature as a mixture of  $\alpha$  and  $\beta$  stereoisomeric forms
- Thujone was nominated by the National Cancer Institute (NCI) based on concerns of:
  - Widespread exposure
  - Lack of toxicity and carcinogenicity data



#### **Human Exposure**

- Uses of Thujone include herbal medicines, food and beverage flavorings, cosmetic products and repellants
- Direct use of Thujone as a food additive banned in the U.S., although certain herbs (e.g. sage) that contain Thujone have Generally Recognized As Safe (GRAS) status
- Concentration allowed in the European Union (EU): 35 mg/kg in alcoholic beverages made from Artemisia species; 0.5 mg/kg in nonalcoholic beverages
- Estimated 97.5<sup>th</sup> percentile intake from France and Britain is 44.3 and 14.2 μg/kg/d, respectively



## **Thujone Activity**

- α-Thujone is the principal component of Absinthe
  - Associated with convulsions, hallucinations, and mental deterioration
  - Widely banned in early 1900s, Absinthe allowed in European Union in early 1990s and now available over the Internet
- Thujone administered to rodents induces seizures
- α-Thujone is a GABA<sub>A</sub> receptor antagonist
  - GABA is the chief inhibitory neurotransmitter in the central nervous system
  - GABA antagonists cause muscle spasms and convulsions





# **Study Rationale and Objectives**

- Objective: characterize the toxicity and carcinogenic activity of Thujone in male and female F344/N rats and B6C3F1 mice
- · Study design:
  - Genetic toxicity studies (in vitro and in vivo)
  - Subchronic gavage studies in  $\alpha\text{-}$  and  $\alpha,\beta\text{-}Thujone$
  - Toxicokinetic studies
  - Chronic gavage studies in  $\alpha,\beta$ -Thujone



## Two week studies of $\alpha$ -Thujone and $\alpha$ , $\beta$ -Thujone

- $\alpha$ -Thujone and an  $\alpha,\beta$ -Thujone mixture were evaluated
- 0, 1, 3, 10, 30, and 100 mg/kg/d administered via gavage (aqueous 0.5% methyl cellulose)
- · Two week studies in rats:
  - α: Female survival decreased in the 100 mg/kg/d group; seizures observed in females
  - α,β: Survival unaffected; seizures not observed
- · Two week studies in mice:
  - α: Male and female survival decreased in 100 mg/kg/d group; seizures observed in males
  - α,β: Male and female survival decreased in 100 mg/kg/d group; seizures not observed



## Three month studies of $\alpha,\beta$ -Thujone

- α,β-Thujone selected for testing since it represents common human exposure
- · Three month study in rats:
  - 0, 12.5, 25, 50, 75, 100 mg/kg/d via gavage
  - Seizures observed in ≥50 mg/kg/d males and ≥25 mg/kg/d females
  - Survival decreased in the 75 and 100 mg/kg/d males and females
  - Brain, pituitary gland, lung, spleen (male), thymus, kidney (female) lesions present
  - Extended diestrus in 50 mg/kg/d female rats
- · Three month study in mice:
  - 0, 6.25, 12.5, 25, 50, 75 mg/kg/d via gavage
  - Seizures observed in male ≥50 mg/kg/d and female ≥25 mg/kg/d groups
  - Survival decreased in 50 and 75 mg/kg/d males and females
  - Lung lesions present in males and females



## **Genetic Toxicology of Thujone**

- Bacterial assays (Salmonella and E. Coli) negative for  $\alpha\text{-}$  and  $\alpha,\beta\text{-}$  Thujone
- $\alpha,\beta\text{-Thujone}$  did not increase micronucleated erythrocytes in male mice after 3 month exposure
- $\alpha, \beta$ -Thujone increased micronucleated erythrocytes in female mice after 3 month exposure
  - Increase in female 50 mg/kg/d group
  - Significant positive trend



# Dose Selection for Chronic $\alpha,\beta$ -Thujone Exposure

- Dose levels selected for chronic exposure (2 year):
  - 0, 12.5, 25, and 50 mg/kg/d for rats:
    - · No decreased survival or limiting pathology observed in 50 mg/kg/d group
    - Mild and transient seizures observed in 50 mg/kg/d group considered not likely to affect survival
  - 0, 3, 6, 12, and 25 mg/kg/d for mice:
    - · No decreased survival or limiting pathology observed in 25 mg/kg/d group
    - Mild and transient seizures observed in 25 mg/kg/d group considered not likely to affect survival
    - Fourth group added since exact dose difficult to determine in 3 month study

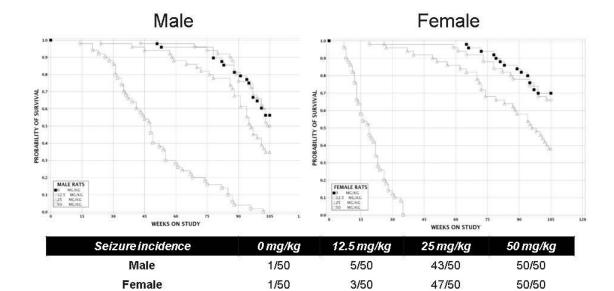


# Pharmacokinetics of $\alpha$ -Thujone

- $\alpha$ -Thujone kinetics evaluated after single administering  $\alpha$  or  $\alpha/\beta$ -thujone in male and female B6C3F1 mice and F344/N rats via IV and oral routes
  - $\,\alpha\text{-Thujone}$  was rapidly eliminated in mice (20-30 min) and rats (2-3 hr)
  - α-Thujone bioavailability was lower in mice than rats
  - $\alpha\mbox{-Thujone}$  concentration was greater in the brain compared to plasma (female rat > male rat)



## F344/N Rat Survival and Seizure Incidence



Weight loss in males and females ≤10%



# Preputial Gland Neoplasm Incidence in Male F344/N Rats

Preputial Gland	0 mg/kg	12.5 mg/kg	25 mg/kg 4/50 5/50 9/50* (18%)	
Adenoma	2/49	1/49		
Carcinoma	1/49*	0/49		
Adenoma or Carcinomaª	3/49*	1/49		

 $<sup>^*</sup>p$  < 0.05;  $^*$  in control group indicates statistically significant trend.  $^a$  Historical Control: same route 6.1% (6/99; 6,6%), all routes 5.2% (68/1295; 0-12%)



# Pheochromocytoma Incidence in Male F344/N Rats

Pheochromocytoma	0 mg/kg	12.5 mg/kg	25 mg/kg 12/49* (24%) 1/49 13/49* (27%)	
Benign <sup>a</sup>	6/50*	8/50		
Malignant	0/50	0/50		
Benign or malignant <sup>b</sup>	6/50*	8/50		

 $<sup>^{\</sup>circ}p < 0.05;^{*}$  in control group indicates statistically significant trend.  $^{\circ}$  Historical Control: same route 13.0% (13/100; 12,14%), all routes 14.1% (183/1295; 6-22%)  $^{\circ}$  Historical Control: same route 14.0% (14/100; 12,16%), all routes 16.1% (208/1295; 6-26%)

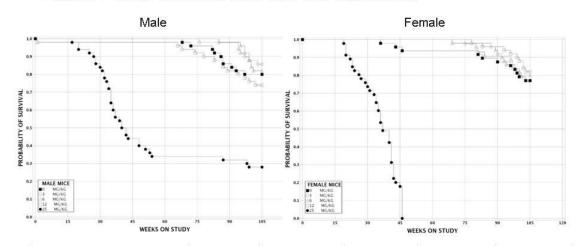


## Selected Non-Neoplastic Lesion Incidences in F344/N Rats

- Brain necrosis increased in 50 mg/kg/d males and brain pigmentation increased in 50 mg/kg/d males and females
- Spleen pigmentation increased in males (25 and 50 mg/kg/d) and females (50 mg/kg/d)
- Pituitary non-neoplastic lesions increased in males (50 mg/kg/d) and females (25 and 50 mg/kg/d)
- · Kidney mineralization increased in males at all doses



## **B6C3F1** Mice Survival and Seizure Incidence



SeizureIncidence	0 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Male	0/50	0/50	0/50	0/50	41/50
Female	1/50	1/50	0/50	0/50	50/50

Weight loss in ≤12 mg/kg/d males and females <5%



## **Thujone Chronic Study Conclusions**

- Male F344/N rats:
  - Some evidence of carcinogenic activity: Increase incidence of preputial gland neoplasms
  - Equivocal evidence of carcinogenic activity: Increased incidence of benign pheochromocytoma of the adrenal medulla may have been related to administration
- Female F344/N rats: No evidence of carcinogenic activity
- · Male and Female B6C3F1 Mice: No evidence of carcinogenic activity



## **Questions/Comments**